09672372 98149643 PMID: 9489999

Carbamyl-phosphate synthetase domain of the **yeast multifunctional protein** Ura2 is necessary for aspartate transcarbamylase inhibition by UTP.

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In Saccharomyces cerevisiae, the first two reactions of pyrimidine biosynthesis are catalyzed by the multifunctional protein Ura2 carrying both carbamyl-phosphate synthetase (CPSase) and aspartate transcarbamylase (ATCase) enzyme activities. In order to study how UTP regulates both of these activities mutant strains were constructed: one strain which expressed the Ura2 protein fused to the green fluorescent protein, and two strains expressed truncated Ura2 proteins. These strains exhibited a phenotype associated with a modified regulation of the pyrimidine pathway. Results presented in this report provide arguments in favor of a single UTP binding site located on the CPSase domain, and support a model in which ATCase activity is inhibited by UTP only when it can interact with the CPSase domain.

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The leaf peroxisomal form (MFP IV) of multifunctional protein functioning in fatty-acid beta-oxidation.
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JOURNAL: Planta (Heidelberg) 196 (4):p642-646 1995
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ABSTRACT: We have purified for the first time from green leaves a multifunctional protein (MFP) involved in fatty acid beta-oxidation. The protein, designated MFP IV, was extracted from green leaves of three-week-old cucumber (Cucumis sativus L.) plants. Chromatography on cation exchanger, separation on hydroxylapatite, and fast-protein liquid chromatography on Phenylsuperose led to a more than 7000-fold purification and to the isolation of an apparently homogeneous 80-kDa monomeric protein. This protein is immunologically related to the glyoxysomal MFP II, as evidenced by immunodecoration with antiserum raised against MFP II. Comparison of molecular masses of all MFPs presently known revealed that the MFP prepared from green leaves (MFP IV) is distinct from MFP II (76.5 kDa) and MFP I (74 kDa) from dark-grown cotyledons. By including other properties in this comparison, we demonstrated that MFP IV can also be distinguished from the glyoxysomal MFP III (81 kDa) and the bacterially **expressed** MFP-a (80 kDa). Moreover, MFP IV is a constituent of leaf peroxisomes and contains the activities of 2-enoyl-CoA hydratase (EC 4.2.1.17), L-3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35) and 3-**hydroxyacyl-CoA epimerase**.

1995

9/3,AB/6 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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07840740 BIOSIS NO.: 000092110906
MITOCHONDRIAL 3-2 TRANS ENOYL-COENZYME A ISOMERASE PURIFICATION CLONING **EXPRESSION** AND MITOCHONDRIAL IMPORT OF THE KEY ENZYME OF UNSATURATED FATTY ACID BETA-OXIDATION
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JOURNAL: BIOL CHEM HOPPE-SEYLER 372 (8). 1991. 613-624. 1991
FULL JOURNAL NAME: Biological Chemistry Hoppe-Seyler
CODEN: BCHSE
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: 3-2trans-Enoyl-Coa isomerase (EC 5.3.3.8) is a key enzyme in mitochondrial .beta.-oxidation of unsaturated fatty acids in bacteria, plant and animal cells. The enzyme was isolated from rat liver mitochondria and purified to homogeneity by two chromatographic steps. Partial polypeptide sequences of the 29 kDa protein were derived from cyanogen bromide, tryptic, Lys-C, and protease V8 fragments by Edman

degradation. Peptide-derived synthetic oligonucleotides were used for the isolation of a 990 bp long isomerase-specific cDNA from rat liver cDNA libraries. 867 bp encode the 289 amino-acid residues of the preisomerase with a molecular mass of 32254 Da. The 1.3-kb mRNA is most strongly **expressed** in skeletal muscle followed by liver, heart, kidney, and weakly **expressed** in spleen and brain. In vivo transcription and translation yielded a 32 kDa polypeptide which was immunoprecipitated by anti rat isomerase antibodies. In the presence of mitochondria the 32 kDa precursor isomerase was processed during mitochondrial import to the 29 kDa mature form of the 3-2trans-enoyl-CoA isomerase with 264 amino-acid residues (Mr 29,706). A N-terminal signal sequence of 25 amino-acid residues directs the import into the mitochondrial matrix and is cleaved in two successive steps passing through an intermediate form of Mr 30475. The two cysteine residues in positions 143 and 148 of the preisomerase are present as free thiol groups as shown by derivatization of the mature, native protein with the fluorescent label N-(iodoacetaminoethyl)-1-naphthylamine-5-sulfonic acid. The mitochondrial 3-2trans enoyl-CoA isomerase shows significant homology and conserved aminoacid exchanges with the mitochondrial enoyl-CoA hydratase, the N-terminal domain of the bifunctional peroxisomal enoyl-CoA-hydratase:3-hydroxyacyl-CoA dehydrogenase and to extended domains of the .alpha.-subunit of the procaryotic .beta.-oxidation complex sharing enoyl-CoA isomerase, D(-)3-**hydroxyacyl-CoA epimerase**, enoyl-CoA hydratase and L(+)3-hydroxyacyl-CoA dehydrogenase activity, encoded by the fad B operon of E. coli.

1991

11195348 20532791 PMID: 11080293
Analysis of the alternative pathways for the beta-oxidation of unsaturated fatty acids using transgenic plants synthesizing polyhydroxyalkanoates in peroxisomes.
Allenbach L; Poirier Y
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Plant physiology (UNITED STATES) Nov 2000, 124 (3) p1159-68, ISSN 0032-0889 Journal Code: P98
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Degradation of fatty acids having cis-double bonds on even-numbered carbons requires the presence of auxiliary enzymes in addition to the enzymes of the core beta-oxidation cycle. Two alternative pathways have been described to degrade these fatty acids. One pathway involves the participation of the enzymes 2, 4-dienoyl-coenzyme A (CoA) reductase and Delta(3)-Delta(2)-enoyl-CoA isomerase, whereas the second involves the epimerization of R-3-hydroxyacyl-CoA via a 3-**hydroxyacyl-CoA epimerase** or the action of two stereo-specific enoyl-CoA hydratases. Although degradation of these fatty acids in bacteria and mammalian peroxisomes was shown to involve mainly the reductase-isomerase pathway, previous analysis of the relative activity of the enoyl-CoA hydratase II (also called R-3-hydroxyacyl-CoA hydro-lyase) and 2,4-dienoyl-CoA reductase in plants indicated that degradation occurred mainly through the epimerase pathway. We have examined the implication of both pathways in transgenic Arabidopsis **expressing** the polyhydroxyalkanoate synthase from Pseudomonas aeruginosa in peroxisomes and producing polyhydroxyalkanoate from the 3-hydroxyacyl-CoA intermediates of the beta-oxidation cycle. Analysis of the polyhydroxyalkanoate synthesized in plants grown in media containing cis-10-heptadecenoic or cis-10-pentadecenoic acids revealed a significant contribution of both the reductase-isomerase and epimerase pathways to the degradation of these fatty acids.

9/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09485776 94327621 PMID: 8051146
Domains of the tetrafunctional protein acting in glyoxysomal fatty acid beta-oxidation. Demonstration of epimerase and isomerase activities on a peptide lacking hydratase activity.
Preisig-Muller R; Guhnemann-Schafer K; Kindl H
Department of Biochemistry, Philipps-University, Marburg, Federal Republic of Germany.
Journal of biological chemistry (UNITED STATES) Aug 12 1994, 269 (32) p20475-81, ISSN 0021-9258 Journal Code: HIV
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Peroxisomes from different eukaryotic organisms house a multifunctional protein acting in fatty acid beta-oxidation. In plant glyoxysomes, one of the isoforms of this protein contains the activities of L-3-hydroxyacyl-CoA hydrolyase (EC 4.2.1.17), L-3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.211), D-3-**hydroxyacyl-CoA epimerase** , and delta 3,delta 2-enoyl-CoA isomerase (EC 5.3.3.8). This was demonstrated after molecular cloning of a cDNA coding for a protein of 79047 Da and its bacterial **expression** . Chromatographic purification yielded a monomeric protein

exhibiting all four activities. In addition, mutant forms were prepared, and peptides representing single domains were purified. Peptides containing the N-terminal region showed D-3-**hydroxyacyl-CoA epimerase** and delta 3,delta 2-enoyl-CoA isomerase activities but lacked 2-trans-enoyl-CoA hydratase and L-3-hydroxyacyl-CoA dehydrogenase activities. Using the N-terminal fragment, we demonstrated that the D-3-hydroxyacyl-CoA converting activity is actually an epimerase rather than part of a combined water eliminating and water attaching system. The C-terminal half of the multifunctional protein represents the dehydrogenase domain.

9/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07465666 92068378 PMID: 1958319
Mitochondrial 3-2trans-Enoyl-CoA isomerase. Purification, cloning, **expression**, and mitochondrial import of the key enzyme of unsaturated fatty acid beta-oxidation.
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Biological chemistry Hoppe-Seyler (GERMANY) Aug 1991, 372 (8) p613-24, ISSN 0177-3593 Journal Code: AHC
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
3-2trans-Enoyl-CoA isomerase (EC 5.3.3.8) is a key enzyme in mitochondrial beta-oxidation of unsaturated fatty acids in bacteria, plant and animal cells. The enzyme was isolated from rat liver mitochondria and purified to homogeneity by two chromatographic steps. Partial polypeptide sequences of the 29 kDa protein were derived from cyanogen bromide, tryptic, Lys-C, and protease V8 fragments by Edman degradation. Peptide-derived synthetic oligonucleotides were used for the isolation of a 990 bp long isomerase-specific cDNA from rat liver cDNA libraries. 867 bp encode the 289 amino-acid residues of the preisomerase with a molecular mass of 32,254 Da. The 1.3-kb mRNA is most strongly **expressed** in skeletal muscle followed by liver, heart, kidney, and weakly **expressed** in spleen and brain. In vitro transcription and translation yielded a 32 kDa polypeptide which was immunoprecipitated by anti rat isomerase antibodies. In the presence of mitochondria the 32 kDa precursor